NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for *o*-NITROANISOLE

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NTP Report on Carcinogens Listing for *o*-Nitroanisole

Carcinogenicity

o-Nitroanisole is *reasonably anticipated to be a human carcinogen* based on evidence in experimental animals (NTP, 1993). When administered in the diet to male and female rats, *o*-nitroanisole induced increased incidences of mononuclear cell leukemia, neoplasms of the urinary bladder, kidney, and large intestine. When administered in the diet to male and female mice, *o*-nitroanisole induced increased incidences of benign and malignant hepatocellular neoplasms in males and increased incidences of hepatocellular adenomas in females.

There are no adequate data available to evaluate the carcinogenicity of *o*-nitroanisole in humans.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

o-Nitroanisole is genotoxic in a wide variety of assays in bacteria and mammalian cells and mutagenic and carcinogenic metabolites have been described (IARC, 1996; NTP, 1993).

No data are available that would suggest that the mechanisms thought to account for tumor induction by *o*-nitroanisole in experimental animals would not also operate in humans.

Listing Criteria from the Report on Carcinogens, Eighth Edition

Known To Be A Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated To Be A Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias or confounding factors, could not adequately be excluded, or

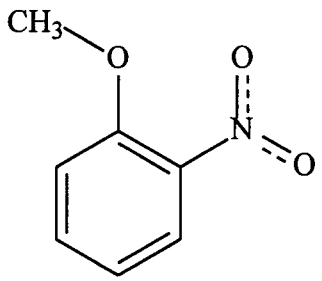
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen or reasonably anticipated to be human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

1.0 INTRODUCTION

o-Nitroanisole
[91-23-6]



1.1 Chemical Identification

o-Nitroanisole ($C_7H_7NO_3$, mol. wt. = 153.14) is also called:

Benzene, 1-methoxy-2-nitro- (9CI)
Anisole, *o*-nitro- (8CI)
Anisole, 2-nitro-
1-Methoxy-2-nitrobenzene
2-Methoxynitrobenzene
NCI-C60388
2-Nitroanisole
1-Nitro-2-methoxybenzene
o-Nitrophenyl methyl ether
ONA

1.2 Physical-Chemical Properties

Property	Information	Reference
Color	Colorless to Yellowish	Budavari (1996)
Physical State	Liquid	Budavari (1996)
Melting Point, °C	9.4	Budavari (1996)
Boiling Point, °C	277	Budavari (1996)
Density at 20 °C	1.254	Lide (1993; cited by IARC, 1996)
Solubility:		
Water at 20 °C	Insoluble	Budavari (1996)
Organic Solvents	Soluble in ethyl alcohol and diethyl ether	Budavari (1996)
Partition Coefficients:		
Log octanol/water	1.73	Hansch et al. (1995; cited by IARC, 1996)
Vapor pressure at 30 °C, Pa	4	BUA (1987; cited by IARC, 1996)

1.3 Identification of Structural Analogues and Metabolites

Structural analogues and metabolites discussed in this report include the following:

o-Anisidine (OND, C_7H_9NO, mol. wt. = 123.15)
o-Nitrophenol (ONP, $C_6H_5NO_3$, mol. wt. = 139.11)
o-Nitrophenyl sulfate ($C_6H_5NO_6S$, mol. wt. = 219.17)
o-Nitrophenyl glucuronide

o-Anisidine is practically insoluble in water, but is miscible in ethanol, alcohol, and diethyl ether. *o*-Nitrophenol is soluble in cold water, and freely soluble in hot water, alcohol, benzene, ether, carbon disulfide, and alkali hydroxides (Budavari, 1996). Structures for at least some of these analogues may be found in Figure 6-1.

1.4 Report Organization

The rest of this report is organized into six additional sections (2.0 Human Exposure, 3.0 Human Studies, 4.0 Mammalian Carcinogenicity, 5.0 Genotoxicity, 6.0 Other Relevant Studies, and 7.0 References) and two appendixes. Appendix A describes the literature search in online databases, and Appendix B provides explanatory information for Figure 5-1.

2.0 HUMAN EXPOSURE

2.1 Use

o-Nitroanisole is used primarily as a precursor to *o*-anisidine, which is prepared by direct nitro-reduction. *o*-Anisidine is used extensively in the synthesis of azo dyes either directly after being converted to a diazonium salt or as a precursor for the preparation of dianisidine, which is diazotized with nitrous acid and coupled. Directly or indirectly, *o*-anisidine is used in the manufacture of over 100 azo dyes (NTP, 1993). *o*-Nitroanisole has also been used as an intermediate for pharmaceuticals (IARC, 1996).

2.2 Production

o-Nitroanisole is produced by treating 2-chloronitrobenzene with sodium methoxide under heat and pressure. The product separates as an oil after dilution with water (IARC, 1996). Of the 5 U.S. plants reporting production or import use of *o*-nitroanisole in the 1977 TSCA Inventory, the 1 known manufacturer reported a production volume of 100,000 to 1,000,000 lb/year (TSCAPP, 1983 update), the 3 known importers did not report volume information, and 1 plant's role was unknown. In 1976 and 1978, imports of the compound through principal U.S. customs districts were reported to total 702,643 and 541,850 lb, respectively (HSDB, 1993). No current data on imports or exports of *o*-nitroanisole were available. Chem Sources (1996) identified 15 U.S. suppliers of *o*-nitroanisole.

2.3 Environmental Exposure

The primary routes of potential human exposure to *o*-nitroanisole are dermal contact, ingestion, and inhalation. Human exposure might be associated with its widespread use in the manufacture of azo dyes (NTP, 1993). *o*-Nitroanisole was not included in the National Occupational Hazard Survey (NIOSH, 1984) or the National Occupational Exposure Survey conducted by NIOSH (NIOSH, 1976).

2.4 Regulations

Atmospheric emissions are regulated by the U.S. EPA under the Clean Air Act. *o*-Nitroanisole is listed in 40 CFR 63 Subpart F (Organic chemical manufacturing industry) and 40CFR 60.489(a) (Stationary source standards). See Appendix C.

3.0 HUMAN STUDIES

No studies were found that evaluated the carcinogenicity of *o*-nitroanisole in humans.

4.0 MAMMALIAN CARCINOGENICITY

4.1 *o*-Nitroanisole

Experimental details of the studies described in this section are presented in Table 4-1.

Summary: There is "sufficient evidence" for the carcinogenicity of *o*-nitroanisole in experimental animals (IARC, 1996). In male mice, there was clear evidence for carcinogenicity with an increased incidence of hepatocellular neoplasms in males administered 666, 2000, or 6000 ppm *o*-nitroanisole in the diet for 103 weeks. Males also had an increased incidence of hepatoblastomas. In females, there was some evidence for carcinogenicity in that only the 2000-ppm dose increased the incidence of hepatocellular neoplasms. In male rats treated with a dietary concentration of 666 or 2000 ppm *o*-nitroanisole and in female rats treated with dietary concentration of 2000 ppm *o*-nitroanisole for 103 weeks, there was an increased incidence of mononuclear-cell leukemia at the end of the treatment period. Increased incidences of tumors of the urinary bladder, large intestine, and kidney occurred in male and female rats treated with 6000 or 18,000 ppm *o*-nitroanisole in their diet for 27 weeks and then maintained on control diet for an additional 77 weeks. These studies also found an overall increased incidence of transitional-cell tumors of the kidney in dosed male and female rats.

4.1.1 Mice

Male B6C3F$_1$ mice orally treated with a dietary concentration of 666, 2000, or 6000 ppm *o*-nitroanisole for 103 weeks showed increased incidences of benign and malignant hepatocellular neoplasms. Female B6C3F$_1$ mice showed an increased incidence of benign and malignant hepatocellular neoplasms in the 2000 ppm dose group (NTP 1993; Irwin et al., 1996). An increased incidence of hepatoblastomas was also observed in treated male mice at the end of the 103-week study.

4.1.2 Rats

Male F344 rats treated with a dietary concentration of 666 or 2000 ppm *o*-nitroanisole and female F344 rats treated with dietary concentration of 2000 ppm *o*-nitroanisole for 103 weeks showed an increased incidence of mononuclear-cell leukemia at the end of the treatment period. Increased incidences of tumors of the urinary bladder, large intestine, and kidney occurred in male and female F344 rats treated with 6000 or 18,000 ppm *o*-nitroanisole in their diet for 27 weeks and then maintained on control diet for an additional 77 weeks (NTP, 1993; Irwin et al., 1996). Interim evaluations were made at 13, 28, 40, and 65 weeks. The overall incidence of urinary bladder neoplasms (including transitional cell carcinoma, squamous cell tumors, and sarcoma) increased in both males and females at 104 weeks. There was a marked increased incidence of large intestine adenomatous polyps in both male and female rats at all

dose levels, and at the high doses, carcinomas of the large intestine occurred in both male and female rats. These studies also found an overall increased incidence of transitional-cell tumors of the kidney in male and female rats when compared to controls.

4.2 *o*-Nitroanisole Metabolite *o*-Anisidine Hydrochloride

Experimental details of the studies described in this section are presented in Table 4-2.

Summary: The salt of a metabolite, *o*-anisidine hydrochloride, was carcinogenic in feeding studies in mice and rats. Urinary bladder neoplasms increased in both mice and rats, while transitional-cell carcinomas in the renal pelvis and thyroid follicular-cell tumors were found in rats only.

4.2.1 Mice

After 103 weeks of a 2500 or 5000 ppm *o*-anisidine hydrochloride treatment orally (in feed) administered to male and female B6C3F$_1$ mice, the National Cancer Institute (NCI, 1978; cited by IARC, 1982) reported that urinary bladder tumors were found in male and female mice receiving the high dose (transitional-cell carcinoma and papilloma) and the low dose (transitional-cell papillomas).

4.2.2 Rats

After 103 weeks of a 5000 ppm or 83 to 88 weeks of a 10,000 ppm *o*-anisidine hydrochloride treatment orally (in feed) administered to male and female F344 rats, the National Cancer Institute (NCI, 1978; cited by IARC, 1982) reported a positive trend in mortality due to the development of urinary bladder tumors in all treated rats killed at 106 to 107 weeks after the initial dose. Thyroid follicular-cell tumors (adenomas, carcinomas, cystadenomas, and cystadenocarcinomas) were found in a dose-related trend in male rats only, while a dose-related trend in transitional-cell carcinomas of the renal pelvis was found in males and females at the high dose.

Table 4-1. Mammalian Carcinogenicity of *o*-Nitroanisole

Age, Strain, and Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose Route	Duration of Exposure	Results/Comments	Reference(s)
Mice							
40-day-old $B6C3F_1$ **(2-Yr Bioassay)**	60M, 60F (per each dose)	60M, 60F (0 ppm in diet)	*o*-nitroanisole, >99%	666, 2000, or 6000 ppm in feed *ad libitum* for up to 103 wk	Groups of 9 or 10 (each sex) from each dose group killed at 15 mo 103-wk treatment	Statistical analysis was performed using logistic regression analysis, life table test, Fisher exact test, or Cochran-Armitage trend test. **Positive** **Liver:** Hepatocellular adenoma increased in both M (LD, 26/50; MD, 41/50; HD, 29/50) compared to controls (14/50) and F (LD, 20/50; MD, 36/50; HD, 18/50) compared to controls (14/50). Increased incidence of hepatocellular adenomas and carcinomas (combined) in M (LD, 33/50; MD, 46/50; HD, 34/50) compared to controls (21/50). Increased incidence of hepatocellular adenoma, carcinoma, or hepatoblastomas in F (LD, 22/50; MD, 37/50; HD, 20/50) compared to controls (17/50). Increased incidence of hepatoblastomas in M (LD, 3/50; MD, 17/50; HD, 9/50) compared to controls (0/50). Hemorrhage, Kupffer-cell pigmentation, eosinophilic focus, focal necrosis, and cytolytic alteration (hepatocyte hypertrophy, nuclear enlargement, and eosinophilic staining of cytoplasm) were also increased in some or all dose groups.	Irwin et al. (1996); NTP (1993)

Table 4-1. Mammalian Carcinogenicity of *o*-Nitroanisole (Continued)

Age, Strain, and Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose Route	Duration of Exposure	Results/Comments	Reference(s)
40-day-old $B6C3F_1$ **(2-Yr Bioassay, cont'd.)**	60M, 60F (per each dose)	60M, 60F (0 ppm in diet)	*o*-nitroanisole >99%	666, 2000, or 6000 ppm in feed *ad libitum* for up to 103 wk	Groups of 9 or 10 (each sex) from each dose group killed at 15 mo 103-wk	**Mean body weights:** MD and HD M and F weighed less than controls at the end of the study. **Survival at 2 years:** Did not differ significantly among treated M (LD, 43/50; MD, 39/49; HD, 40/50) compared to controls (35/50). In F, survival in the LD group (26/50) was marginally reduced compared to controls (38/50).	Irwin et al. (1996); NTP (1993)
Rats							
approx. 40-day-old F344/N **(2-Yr Bioassay)**	60M and 60F per dose	60M and 60F (0 ppm in diet)	*o*-nitroanisole, >99%	222, 666 or 2000 ppm in feed *ad libitum* for up to 103 wk	103 wk	**Positive** **Blood:** Increased incidence of mononuclear-cell leukemia seen in M (MD, 42/50; HD, 34/50; $P < 0.001$, life table trend test) compared to controls (26/50) and F (HD, 26/50; $p = 0.024$, life table trend test) compared to controls (14/50). **Kidney:** Nephropathy occurred in all M; the severity increased with exposure level. Focal hyperplasia of the renal tubule epithelium was present in 3 M at LD and 2 M at HD. Renal tubular adenomas occurred in 1 M in HD group and 2 M in HD group had renal tubular carcinomas vs. none in controls. **Urinary bladder:** Transitional cell carcinoma in 1/50 F in HD group vs. none in controls. Transitional hyperplasia found in F (LD, 1/50; HD, 6/50) and M (HD, 2/50).	Irwin et al. (1996); NTP (1993)

Table 4-1. Mammalian Carcinogenicity of *o*-Nitroanisole (Continued)

Age, Strain, and Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose Route	Duration of Exposure	Results/Comments	Reference(s)
approx. 40-day-old F344/N **(2-Yr Bioassay cont'd.)**	60M and 60F per dose	60M and 60F (0 ppm in diet)	*o*-nitroanisole, >99%	222, 666, or 2000 ppm in feed *ad libitum* for up to 103 wk	103 wk Groups of 9 or 10 (each sex) from each dose group were killed at 15 mo (interim evaluation)	**Forestomach:** A dose-related increase of ulcers was seen in M only (0 ppm, 3/50; LD, 3/50; MD, 8/50; HD, 16/50) and a dose-related increase of focal hyperplasia was seen in M (3/50, 16/50, 25/50, 32/50, respectively) and F (8/50, 8/50, 13/50, 28/50, respectively). Squamous cell papillomas were detected in 2 F (LD and HD), and in 2 M (MD and HD). In NTP historical controls, squamous cell papillomas were detected in 2/800 M and 1/800 F. Squamous cell carcinomas were detected in 2 M (MD and HD) and 1 F (HD). In NTP historical controls, squamous cell carcinomas were detected in 1/800 M and none have occurred in F (N = 800). **Body weights:** Similar to those of controls. **Survival at 2 years: Significantly** decreased in M (HD, 9/50) compared to controls (32/50), and was unchanged in F.	Irwin et al. (1996); NTP (1993)

Table 4-1. Mammalian Carcinogenicity of *o*-Nitroanisole (Continued)

Age, Strain, and Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose Route	Duration of Exposure	Results/Comments	Reference(s)
approx. 41-day-old F344/N **(Stop-Exposure Study)**	60M and 60F per dose	60M and 60F (0 ppm in diet)	*o*-nitroanisole >99%	6000 or 18,000 ppm in feed *ad libitum* for 27 wk	27 wk Groups of 1 to 10 rats from each dose group killed at 3, 6, 9, or 15 mo for interim evaluation and the remaining rats killed 77 wk after cessation of treatment (104 wk)	**Positive** **Urinary bladder:** Overall increased incidence of neoplasms at 104 wk. Increased incidence of transitional-cell papilloma in LD M only (LD, 9/59 vs. controls 0/59, [$p < 0.01$, Fisher's exact test]). Dose-related increased incidence of transitional-cell carcinoma in M (LD, 27/59; HD, 50/60 [$p < 0.01$]) compared to controls (0/59) and in F (LD, 28/59; HD, 48/60 ($p < 0.01$) compared to controls (0/58). Squamous-cell papillomas (M, 4/60; F, 4/60 vs. none in controls) and carcinomas (M, 6/60 [$p < 0.05$, Fisher's exact test]; F, 1/60 vs. none in controls) of the urinary bladder occurred in the HD M and F. Incidence of sarcomas increased in M (LD, 2/59; HD, 9/60 [$p < 0.01$]) compared to controls (0/59) and in F (LD, 2/59; HD, 14/60 [$p < 0.01$]) compared to controls (0/58).	Irwin et al. (1996); NTP (1993)

Table 4-1. Mammalian Carcinogenicity of *o*-Nitroanisole (Continued)

Age, Strain, and Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose Route	Duration of Exposure	Results/Comments	Reference(s)
approx. 40-day-old F344/N **(Stop-Exposure Study, cont'd.)**	60M and 60F per dose	59M and 58F (0 ppm in diet)	*o*-nitroanisole >99%	6000 or 18,000 ppm in feed *ad libitum* for up to 27 wk	Groups of 1 to 10 rats from each dose group killed at 3, 6, 9 or 15 mo for interim evaluation and the remaining rats killed 77 wk after cessation of treatment (104 wk	**Positive** **Large intestine:** Increased incidence of adenomatous polyps in M (LD, 26/60; HD, 30/60 vs. control, 0/60) and F (LD, 8/60; HD, 18/60 vs. control, 0/60) ($p \leq 0.01$). Carcinomas detected in 5/60 ($p \leq 0.05$) HD M and 2/60 HD F vs. none in controls. **Kidney:** Transitional-cell papillomas were present in HD M (3/60) and HD F (1/60) vs. none in controls. Transitional-cell carcinomas found in M (LD, 1/60; HD, 6/60 [$p < 0.05$, Fisher's exact test]) and F (HD, 1/60) vs. none in controls. **Body weight:** Markedly lower in treated M and F than in those of controls during the 27-wk treatment period, and did not recover after cessation of treatment. **Survival:** Decreased in treated M and F. Kaplan-Meir probability of survival at end of study being 63% and 68% for control M and F, respectively, 4% and 23% for LD M and F, respectively, and 0% for both HD M and F.	Irwin et al. (1996); NTP (1993)

Abbreviations: bw = body weight; F = females; HD = high dose; LD = low dose; M = males; MD = mid dose

Table 4-2. Mammalian Carcinogenicity of *o*-Nitroanisole Metabolite *o*-Anisidine Hydrochloride

Age, Strain, and Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose Route	Duration of Exposure	Results/Comments	Reference
B6C3F$_1$ mice, 6-wk-old	55M and 55F	55M and 55F per dose	*o*-anisidine hydrochloride, >99%	2500 or 5000 ppm in feed *ad libitum* for up to 103 wk	103 wk; surviving mice were killed at 105 wk	**Positive** **Urinary bladder:** Transitional-cell carcinomas increased in HD M (15/53; $p < 0.001$) vs. controls (0/48) and in HD F (18/50; $p < 0.001$) vs. controls [0/50 F]). Increased incidence of transitional-cell papillomas in M (2/55 LD and 7/53 HD) compared to controls (0/48) and in F (1/51 LD and 4/50 HD) compared to controls (0/50).	NCI (1978; cited by IARC, 1982)
F-344 rats, 6-wk-old	55M and 55F per dose	55M and 55F	*o*-anisidine hydrochloride, >99%	5000 or 10,000 ppm in feed *ad libitum*	5000 ppm for 103 wk, and 10,000 ppm for 83 to 88 wk; surviving rats were killed at 106 to 107 wk	**Positive** **Urinary bladder:** Transitional-cell carcinomas found in a dose-related trend in M (LD, 50/54 [$p < 0.001$], HD, 51/52 [$p < 0.001$]) compared to controls (0/51) and in F (LD, 41/49 [$p < 0.001$]; HD, 50/51 [$p < 0.001$]) compared to controls (0/49). **Renal pelvis:** Transitional-cell carcinomas found in 3/55 LD M ($p < 0.005$) and in 7/53 HD M ($p = 0.006$), but only in 1/54 HD F. Papillary necrosis found in some treated rats. **Urinary tract:** Hydronephrosis and epithelial hyperplasia found in some treated rats. **Thyroid:** Incidence of thyroid follicular-cell tumors (adenomas, carcinomas, cystadenomas, and cystadenocarcinomas) increased, 7/40 LD M ($p = 0.002$), and 6/40 HD M ($p = 0.005$) compared to controls (0/53); no significant increase in the incidence of thyroid tumors in F. **Mortality**: Dose-related trend ($p < 0.001$) was seen for M and F; M and F receiving HD did not survive to termination of study, although 80 to 89% survived past wk 52. Mortality was due to the development of urinary bladder tumors.	NCI (1978; cited by IARC, 1982)

Note: IARC did not specify which statistical test was used in each case.
Abbreviations: bw = body weight; F = females; HD = high dose; LD = low dose; M = males

5.0 GENOTOXICITY

Studies of the genotoxic effects of *o*-nitroanisole are summarized in Table 5-1.

Summary: *o*-Nitroanisole was reported to be positive in a number of prokaryotic and mammalian *in vitro* and *in vivo* test systems [see Genetic Activity Profile, Figure 5-1 (data limited to IARC, 1996)]. *o*-Nitroanisole was found to induce gene mutations in multiple strains of *Salmonella typhimurium,* DNA damage in *Bacillus subtilis,* gene mutations in mouse lymphoma cells, sister chromatid exchanges and chromosomal aberrations (with S9 only) in Chinese hamster ovary (CHO) cells, and DNA damage in human mononuclear blood cells. It did not induce chromosomal aberrations in CHO cells without S9 activation. Unless otherwise specified, rat liver S9 was the source of metabolic activation *in vitro*.

Information for studies reviewed in IARC (1996) was often limited to qualitative data. Pertinent information on study design, doses tested, chemical purity, etc., was generally not provided.

5.1 Noneukaryotic Systems

5.1.1 Gene Mutations

Chiu et al. (1978; cited by IARC, 1996) reported that *o*-nitroanisole was mutagenic in *S. typhimurium* strains TA100 [LED = 20 μmol) and TA98 [LED = 10 μmol) in the absence of metabolic activation. Tokiwa et al. (1981) found that *o*-nitroanisole was weakly positive in *S. typhimurium* strain TA100 in the presence of S9 activation only [LED = 50 μg/plate (0.33 μmol/plate)] but not TA98. Haworth et al. (1983; cited by NTP, 1993) tested *o*-nitroanisole for mutagenic activity in *S. typhimurium* strains TA100, TA98, TA97, TA1535, and TA1537 both with and without 10% rat or hamster S9 activation in the pre-incubation assay. *o*-Nitroanisole was positive in TA100 with and without S9 activation (rat or hamster) and TA1535 without S9 starting at a dose of 333 μg/plate (2.18 μmol/plate). In a similar preincubation study by Shimizu and Yano (1986), *o*-nitroanisole induced a dose-related increase in strains TA98, TA1538, and TA100 without S9 activation at doses of 0.1 to 10 μL/plate (0.8 to 40 μmol/plate) in the pre-incubation assay. *o*-Nitroanisole was negative for mutagenic activity in strains TA1535 and TA1537 in the absence of S9. Studies with metabolic activation were not conducted. Dellarco and Prival (1989) found that *o*-nitroanisole in the presence of S9 gave a clear mutagenic response in strains TA98 and TA100 using the standard plate incorporation protocol, whereas a modified pre-incubation method incorporating 2 mM flavin mononucleotide in the S9 mix to facilitate nitroreduction did not. From the graphical data presented for TA100, the LED is estimated to be 3 μmol/plate. Studies without S9 were not conducted.

5.1.2 DNA Damage

Shimizu and Yano (1986) also reported that *o*-nitroanisole was positive in the rec assay employing *Bacillus subtilis* strains H17 (rec^-) and M45 (rec^+). Dose related differences in the inhibition zone diameter were observed at 0.5 and 5 μL per filter (0.004 to 0.04 μmol/filter).

5.2 Mammalian Systems *In Vitro*

5.2.1 Gene Mutations

Myhr et al. (1985; cited by NTP, 1993) reported that a dose-related increase in mutant colonies was observed in mouse lymphoma L5178Y $tk^{+/-}$ cells (tested only in the absence of S9 activation) beginning at doses where the relative total growth was below 50% [LED = 0.15 μL/mL (0.19 μg/mL or 1.2 μM)].

5.2.2 DNA Damage

Galloway et al. (1987; cited by NTP, 1993) found that *o*-nitroanisole induced a dose-dependent increase in sister chromatid exchanges (SCE) in CHO cells both in the presence [LED = 608 μg/mL (3970 μM)] and absence [LED = 123 μg/mL (800 μM)] of S9 metabolic activation. Doses tested ranged from 12.3 to 350.0 μg/mL (80 to 2290 μM) without S9 and from 608 to 1010 μg/mL (3970 to 6600 μM) with S9.

5.2.3 Chromosomal Damage

Galloway et al. (1987; cited by NTP, 1993) further reported that *o*-nitroanisole induced chromosomal aberrations (preferentially occurring in the long arm of the x-chromosome) in CHO cells in the presence [LED = 1060 μg/mL (6920 μM)] but not the absence [HID = 803 μg/mL (5250 μM)] of S9 metabolic activation. Doses tested ranged from 216.3 to 803.4 μg/mL (1412 to 5250 μM) without S9 activation and from 519.0 to 1060 μg/mL (3390 to 6920 μM) with S9.

5.3 Mammalian Systems *In Vivo* (DNA Damage in Humans)

Hengstler et al. (1995) reported on a limited human epidemiology study evaluating DNA damage via alkaline elution in the mononuclear blood cells of 16 fire fighters exposed to 27.8% *o*-nitroanisole (along with other chemicals) for up to 8 hours during a chemical plant fire. At 19 days after exposure, the rate of DNA elution was statistically increased in cells sampled from the fire fighters over that in two concurrently sampled control groups. At 88 days after exposure, the elution rate was not different from that in the concurrently sampled control populations.

Table 5-1. Summary of *o*-Nitroanisole Genotoxicity Studies

Test System	Biological Endpoint	S9 Metabolic Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
5.1 Noneukaryotic Systems							
5.1.1 Gene Mutations							
Salmonella typhimurium strains TA100 and TA98	*his* gene mutations	-	n.p.	n.g.	positive	LED = 10 µmol (TA98), 20 µmol (TA100)	Chiu et al. (1978; cited by IARC, 1996)
S. typhimurium strains TA100 and TA98	*his* gene mutations	+/-	n.p.	50 to 100 µg/plate (0.33 - 0.65 µmol/plate)	positive/ negative	Weakly mutagenic for only strain TA100 in the presence of S9. LED = 50 µg/plate (0.33 µmol/plate)	Tokiwa et al. (1981)
S. typhimurium strains TA100, TA98, TA97, TA1535, and TA1537	*his* gene mutations, pre-incubation assay	+/- 10% rat or hamster	n.p.	10 to 3333 µg/plate (0.065 - 21.77 µmol/plate)	positive/ positive	Positive responses were noted for strains TA100 with and without S9 and TA1535 without S9. LED = 333 µg/plate (2.18 µmol/plate)	Haworth et al. (1983; cited by NTP, 1993)
S. typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538	*his* gene mutations, pre-incubation assay	-	98%	0.1 to 10 µL/plate (0.8 - 80 µmol/plate)	positive	Dose related increase in revertants/plate in strains TA98, TA1538, and TA100. LED = 1.0 µL/plate (8 µmol/plate)	Shimizu and Yano (1986; cited by IARC, 1996)
S. typhimurium strains TA98 and TA100	*his* gene mutations, modified pre-incubation assay with 2 mM FMN in the S9 to facilitate nitro reduction	+ 20% rat or hamster S9	n.p.	0.3 to 10 µmol/plate	positive	The standard plate incorporation protocol gave a clear mutagenic response while the modified pre-incubation method did not. LED ≈ 3.0 µmol/plate.	Dellarco and Prival (1989; cited by IARC, 1996)
5.1.2 DNA Damage							
Bacillus subtilis strain H17 (rec^-) and M45 (rec^+)	rec assay (DNA damage induced differential toxicity)	-	98%	0.5 and 5.0 µL/filter (4 - 40 µmol/filter)	positive	Dose related increase in the inhibition zone diameter. LED = 0.5 µL/filter (4 µmol/filter)	Shimizu and Yano (1986; cited by IARC, 1996)

Table 5-1. Summary of *o*-Nitroanisole Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metabolic Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
5.2 Mammalian Systems In Vitro							
5.2.1 Gene Mutations							
mouse lymphoma L5178Y cells	*tk* gene mutations	-	n.p.	0.0125 to 0.5 µL/mL (0.102 - 4 mM)	positive	A dose- related increase in mutant colonies was observed at doses where the relative total growth was below 50%. LED = 0.15 µL/mL (1.2 µM)	Myhr et al. (1985; cited by NTP, 1993)
5.2.2 DNA Damage							
Chinese hamster ovary (CHO) cells	sister chromatid exchanges	+/-	n.p.	+S9: 608 to 1010 µg/mL (3970 – 6600 µM); -S9: 12.3 to 350.0 µg/mL (80 – 2290 µM)	positive/ positive	At higher doses [above 811 µg/mL (5300 µM)] a delayed harvest was used to offset induced cell cycle delay. +S9 LED = 608 µg/mL (3970 µM); -S9 LED = 123 µg/mL (800 µM).	Galloway et al. (1987; cited by NTP, 1993)
5.2.3 Chromosomal Damage							
CHO cells	chromosomal aberrations	+/-	n.p.	+S9: 519.0 to 1060 µg/mL (3390 – 6920 µM); -S9: 216.3 to 803.4 µg/mL (1410-5250 µM)	positive/ negative	Significant increase in CA at the highest dose +S9 mainly involved breaks in the long arm of the X chromosome. +S9 LED = 1060 µg/mL (6920 µM); -S9 HID = 803.4 µg/mL (5250 µM).	Galloway et al. (1987; cited by NTP, 1993)
5.3 Mammalian Systems *In Vivo* (DNA Damage in Humans)							
16 exposed fire fighters	DNA damage measured by alkaline elution in mononuclear blood cells	NA	n.p.	27.8% for up to 8 hours, blood collected 19 days and 3 months post exposure	positive	The rate of elution was statistically increased in cells sampled from the fire fighters 19 days after exposure over that in two concurrently sampled control groups at 19 days. At 88 days after exposure, the elution rate was not different from that in the concurrently sampled control populations.	Hengstler et al. (1995)

Abbreviations: bw = body weight; HID = highest ineffective dose; LED = lowest ineffective dose; NA = not applicable; n.g. = not given; n.p. = not provided
The density of *o*-nitroanisole is 1.254 g/mL, which is equivalent to 8.189 µmol/µL.

Figure 5-1. Genetic Activity Profile of *o*-Nitroanisole (Data limited to IARC, 1996)

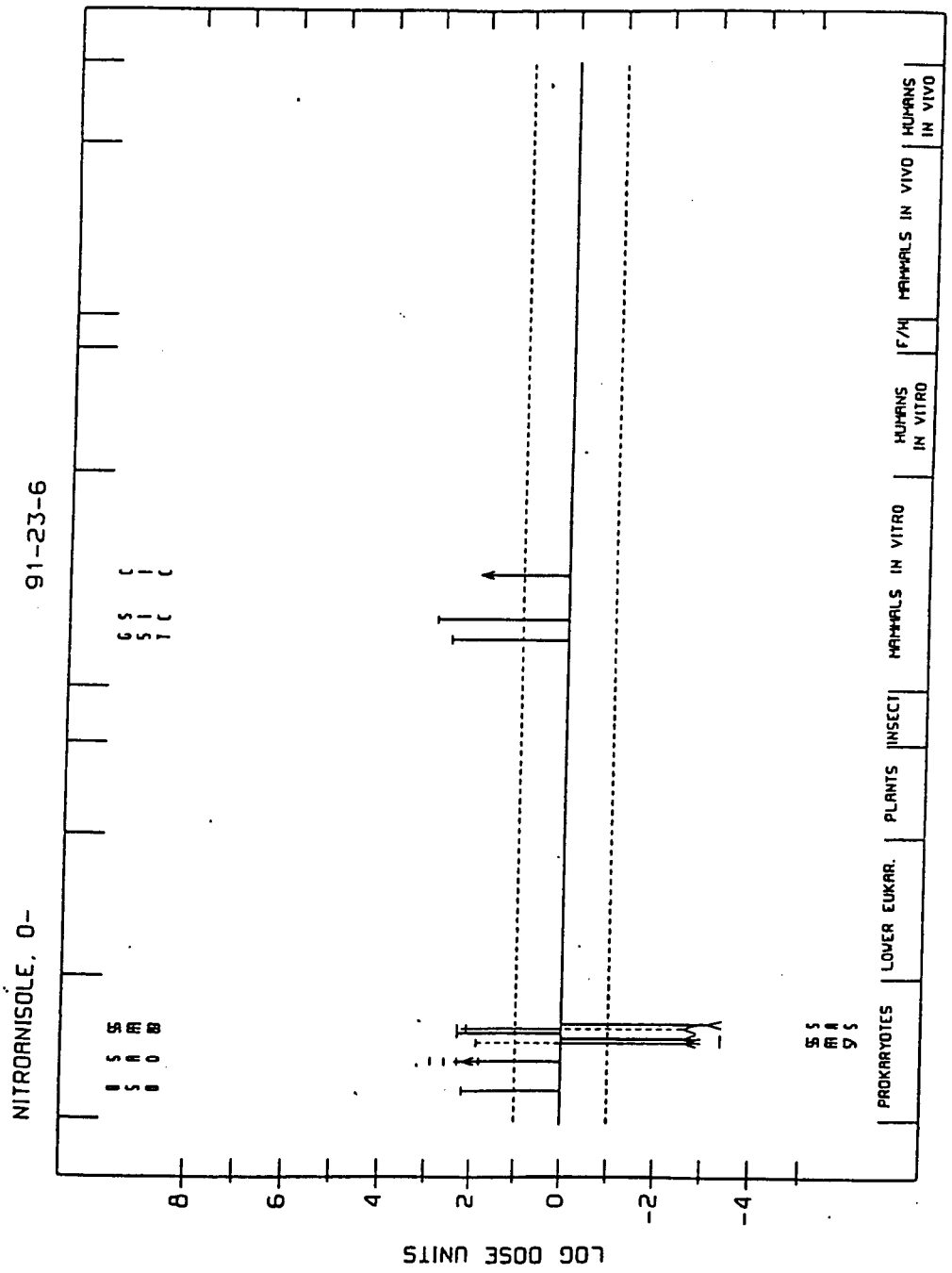
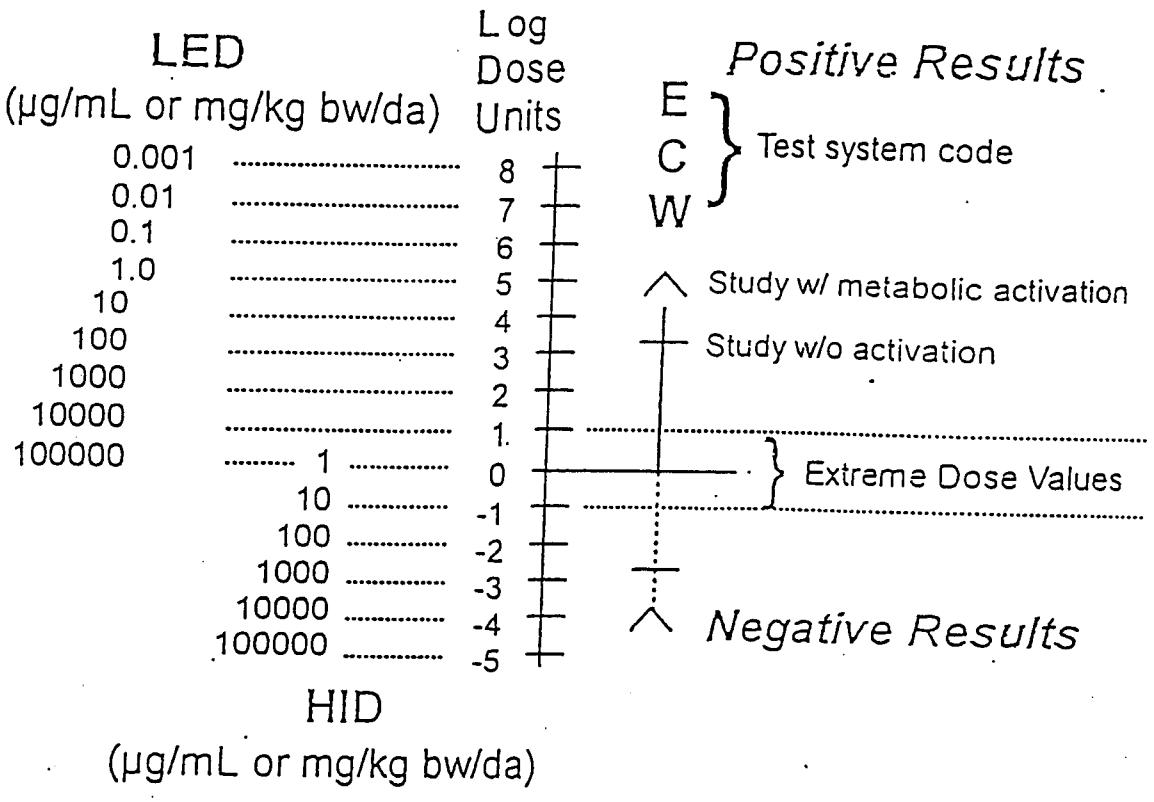


Figure 5-2. Schematic View of a Genetic Activity Profile (GAP)



A schematic view of a Genetic Activity Profile (GAP) representing four studies (two positive and two negative) for an example short-term test, ECW. Either the lowest effective dose (LED) or the highest ineffective dose (HID) is recorded from each study, and a simple mathematical transformation (as illustrated above) is used to convert LED or HID values into the logarithmic dose unit (LDU) values plotted in a GAP. For each test, the average of the LDUs of the majority call is plotted using a solid vertical bar drawn from the origin. A dashed vertical bar indicates studies that conflict with the majority call for the test. Note in cases where there are an equal number of positive and negative studies, as shown here, the overall call is determined positive. The GAP methodology and database have been reported previously (Garrett et al., 1984; Waters et al., 1988, 1991).

Garrett, N.E., H.F. Stack, M.R. Gross, and M.D. Waters. 1984. An analysis of the spectra of genetic activity produced by known or suspected human carcinogens. Mutat. Res. 143:89-111.

Waters, M.D., H.F. Stack, A.L. Brady, P.H.M. Lohman, L. Haroun, and H. Vainio. 1988. Use of computerized data listings and activity profiles of genetic and related effects in the review of 195 compounds. Mutat. Res. 205:295-312.

Waters, M.D., H.F. Stack, N.E. Garrett, and M.A. Jackson. 1991. The genetic activity profile database. Environ. Health Perspect. 96:41-45.

6.0 OTHER RELEVANT STUDIES

6.1 Absorption, Distribution, Metabolism, and Excretion

Summary: [^{14}C]*o*-Nitroanisole was readily absorbed into the blood of rats orally administered 50 mg/kg bw (326 μmol/kg bw), but saturation occurred when they were administered 500 mg/kg bw (3260 μmol/kg bw). After oral administration of 5, 50, or 500 mg/kg bw (33, 326, or 3260 μmol/kg bw) [^{14}C]*o*-nitroanisole, the majority of the dose was excreted in the urine of the rats within 24 (low- and mid-doses) or 48 (high-dose) hours. After 7 days, less than 0.5% of the original dose was detected in the rat carcasses. Elimination of the dose was mainly via metabolism of *o*-nitroanisole to *o*-nitrophenol, followed by sulfation to *o*-nitrophenyl sulfate and glucuronidation to *o*-nitrophenyl glucuronide. *o*-Anisidine was also detected in the urine of rats orally administered *o*-nitroanisole, indicating that *O*-demethylation was the major metabolic pathway to form *o*-nitrophenol, followed by sulfate or glucuronide conjugation. When rats were injected intravenously (i.v.) with 25 mg/kg bw (163 μmol/kg bw) [^{14}C]*o*-nitroanisole, the distribution of ^{14}C to all tissues was rapid, with peak concentrations reached at 15 min after administration of the dose. Subsequently, the dose was rapidly eliminated, with less than 1% of the dose present in all tissues at 8 h. A metabolite profile similar to that observed for oral administration was found 24 h after i.p. administration of 25 mg/kg bw (163 μmol/kg bw). Within 7 days, 86% of the i.p. dose was eliminated in the urine, with 82% excreted within 24 h. Fecal excretion was similar to that found after oral administration.

The absorption, distribution, metabolism, and excretion of [^{14}C]*o*-nitroanisole were studied in male Fischer 344 (F-344) rats by Miller et al. (1985). Rats (3 per dose group) were orally administered a single dose of 5, 50, or 500 mg/kg bw (33, 326, or 3260 μmol/kg bw) [^{14}C]*o*-nitroanisole (purity >98%), and the ^{14}C activity in excreta was analyzed daily for 7 days. After oral administration of either 50 mg/kg bw or 500 mg/kg bw (326 or 3260 μmol/kg bw) [^{14}C]*o*-nitroanisole to F-344 rats (3 per dose group), experiments were conducted to determine *o*-nitroanisole concentrations in blood and stomach contents of treated animals. Less than 10% of the initial dose of 50 mg/kg bw (326 μmol/kg bw) remained in the stomach at 6 h compared to 36% of the initial 500 mg/kg bw (3260 μmol/kg bw) dose, indicating that *o*-nitroanisole was readily absorbed at the 50 mg/kg bw (326 μmol/kg bw) dose level but that saturation had taken place at the 500 mg/kg bw (3260 μmol/kg bw) dose.

Analysis of excreta for up to 7 days showed that 7% (~5% within 48 h) of the dose was excreted in the feces at all dose levels. The majority of the dose was excreted in the urine within 24 h for the low-dose and mid-dose groups (73% of the 5 mg/kg and 69% of the 50 mg/kg dose) and within 48 h for the high-dose group (71% of the 500 mg/kg dose). The carcasses of rats sacrificed 7 days after oral administration of [^{14}C]*o*-nitroanisole contained less than 0.5% of the original dose. The total recovery of administered ^{14}C in tissues, blood, and excreta obtained from orally dosed rats (n = 9) was 80.5 ± 13.7% over the 7-day period.

The 8-h urinary metabolite profiles of rats dosed with 50 or 500 mg/kg bw (326 or 3260 μmol/kg bw) were not substantially different. However, the percentage of the dose excreted was significantly different (48% of the 50 mg/kg [326 μmol/kg] dose vs. 11% of the 500 mg/kg [3260 μmol/kg] dose). Elimination of the dose was predominantly *via* metabolism of *o*-nitroanisole to *o*-nitrophenol (5 to 8% of urinary radioactivity), followed by sulfation to *o*-

nitrophenyl sulfate (64 to 68% of urinary radioactivity) and glucuronidation to *o*-nitrophenyl glucuronide (13 to 15% of urinary radioactivity). Analysis of urinary metabolites also showed the reactive metabolite *o*-anisidine was present (0.6% of urinary radioactivity) at 8 h. These findings suggest that *O*-demethylation is the major metabolic pathway to form *o*-nitrophenol followed by sulfate or glucuronide conjugation.

Since the elimination rate of ^{14}C was saturated at high doses (500 mg/kg bw [3260 μmol/kg bw]) in orally dosed rats, a medium dose was chosen for subsequent i.v. metabolic and pharmacokinetic (section 6.2) studies. Following a 25 mg/kg bw (163 μmol/kg bw) [^{14}C]*o*-nitroanisole i.v. injection into the tail vein of rats, blood, tissues, and excreta were collected at 15 min to 7 days. The distribution of ^{14}C to all tissues was rapid, with peak concentrations (muscle, 20% of the dose; skin, 10%; fat, 6.8%; blood, 6.5%; liver, 4.8%; plasma, 3.1%; kidney, 2.8%; small intestine, 1.9% of the radiolabel) reached at 15 min after administration of the dose. Subsequently, the dose was rapidly eliminated, with less than 1% of the dose present in all tissues at 8 h.

A metabolite profile similar to that observed for oral administration was found 24 h after i.p. administration of 25 mg/kg bw [163 μmol/kg bw]: 63% *o*-nitrophenyl sulfate, 11% *o*-nitrophenyl glucuronide, 1.5%*o*-nitrophenol, 0.6% *o*-anisidine, 16% polar metabolites (unidentified), 5.5% acidic metabolites, and 1.3% basic or neutral metabolites.

Within 7 days, 86% of the dose was eliminated in the urine, with 82% excreted within 24 h. Fecal excretion was similar to that found after oral administration, with 7.5% of the dose excreted within 24 h and 9% by 7 days. Total urinary and fecal excretion accounted for 95 ± 5% of the dose within 7 days.

Biliary excretion of radioactivity closely corresponded to the amount found in the feces and intestinal contents, suggesting no enterohepatic circulation occurred (Miller et al., 1985).

6.2 Pharmacokinetics

Summary: In rats, following a single i.p. dose of 25 mg/kg bw (163 μmol/kg bw) [^{14}C]*o*-nitroanisole, the radiolabel was rapidly distributed to all tissues within 15 min (peak concentrations), with the highest percentage of the dose located in muscle and skin. The ratio of ^{14}C present in muscle and skin to that in blood at all time points was less than 1.0. Rapid elimination occurred in all tissues such that less than 1% of the dose was present in all tissues 8 h after administration of the dose. The elimination of ^{14}C followed first-order biphasic elimination kinetics, with the initial phase having a half-life ($t_{1/2}$) of 1 to 2 h. Plasma, liver, lung, small intestine, and kidney had a terminal elimination $t_{1/2}$ of approximately 2.5 days. Testes, spleen, blood, and muscle had terminal elimination $t_{1/2}$ of 6.2, 5.2, 4.5, and 4.4 days, respectively. The elimination of *o*-nitroanisole from blood followed first-order biphasic elimination kinetics. The initial elimination $T_{1/2}$ was 30 min and the terminal elimination $t_{1/2}$ was 2.2 h. *o*-Nitroanisole was rapidly eliminated from other tissues in a monophasic manner ($t_{1/2}$ = 15 min to 2 h). Urinary metabolites of *o*-nitroanisole were predominantly the conjugated compounds (63% as sulfates and 11% as glucuronides). A minor reductive metabolic pathway of *o*-nitroanisole to *o*-anisidine also occurred in these studies.

The pharmacokinetics of *o*-nitroanisole have been studied by Miller et al. (1985). The experimental procedure was mentioned in section 6.1. Following a single i.p. dose of 25 mg/kg bw (163 μmol/kg bw) [^{14}C]*o*-nitroanisole, the radiolabel was rapidly distributed to all tissues within 15 min (peak concentrations), with the highest percentage of the dose located in muscle and skin, which was thought to be a reflection of their large fraction of total body mass. The ratio of ^{14}C present in muscle and skin to that in blood at all time points was less than 1.0, suggesting that these tissues were not storage sites for *o*-nitroanisole or its metabolites. Rapid elimination occurred in all tissues such that less than 1% of the dose was present in all tissues 8 h after administration of the dose. The elimination of ^{14}C followed first-order biphasic elimination kinetics, with the initial phase having a $t_{1/2}$ of 1 to 2 h. Plasma, liver, lung, small intestine, and kidney had a terminal elimination $t_{1/2}$ of approximately 2.5 days. Testes, spleen, blood, and muscle had terminal elimination $t_{1/2}$ of 6.2, 5.2, 4.5, and 4.4 days, respectively. The terminal elimination decay rate constants for fat and skin could not be accurately determined (Miller et al., 1985).

The elimination of *o*-nitroanisole from blood followed first-order biphasic elimination kinetics. The initial elimination $T_{1/2}$ was 30 min and the terminal elimination $t_{1/2}$ was 2.2 h. *o*-Nitroanisole was rapidly eliminated from other tissues in a monophasic manner ($t_{1/2}$ = 15 min to 2 h).

An uptake phase ($t_{1/2}$ approximately 6 min) prior to the elimination of *o*-nitroanisole was found in skin and adipose tissue. The elimination $t_{1/2}$ of *o*-nitroanisole from skin and adipose tissue was 0.39 and 2.0 h, respectively.

The elimination $t_{1/2}$ for *o*-nitroanisole in urine was 3.6 h, with only 0.5% of the dose excreted as parent *o*-nitroanisole. Parent compound was not detected in the feces at any time point.

Urinary metabolites of *o*-nitroanisole were predominantly the conjugated compounds (63% as sulfates and 11% as glucuronides). A minor reductive metabolic pathway of *o*-nitroanisole to *o*-anisidine also occurred in these studies. This pathway is important to mention due to the carcinogenicity and mutagenicity of *o*-anisidine. The liver and blood had similar pharmacokinetic profiles. However, *o*-anisidine was detected in the liver and not in the blood. *o*-Anisidine concentrations in the liver decreased at a rate that paralleled that of *o*-nitroanisole. Aniline compounds are known to bind to the red blood cell constituents (Bus and Sun, 1979; Bus, 1981; both cited by Miller et al., 1985). Perhaps *o*-anisidine is not seen in blood because of its being sequestered in the red blood cells or because it is further metabolized (Miller et al., 1985).

6.3 Modes of Action

o-Nitroanisole was reported to be positive in the following prokaryotic and mammalian *in vitro* and *in vivo* test systems. *o*-Nitroanisole was found to induce gene mutations in multiple strains of *Salmonella typhimurium,* DNA damage in *Bacillus subtilis,* gene mutations in mouse lymphoma cells, sister chromatid exchanges and chromosomal aberrations (+S9 only) in Chinese hamster ovary (CHO) cells, and DNA damage in human mononuclear blood cells. It did not induce chromosomal aberrations in CHO cells without S9 activation (see section 5.0). Thus, *o*-nitroanisole may induce tumors via a genotoxic mechanism.

6.4 Structure-Activity Relationships

Tennant et al. (1990) used two molecular descriptors—Ashby's potentially alerting substructures (structural alerts; SA) (Ashby, 1985) and estimated electrophilic reactivity (K_e)—in combination to develop a QSAR model to predict the carcinogenicity of 44 chemicals bioassayed by the US National Toxicology Program (NTP, 1993). Of the 44 chemicals, Benigni (1991) found that *o*-nitroanisole was one of 7 compounds predicted to be carcinogenic due to the theoretically estimated K_e values of the chemicals and the presence of SA in the molecules. In the case of *o*-nitroanisole, the SA was identified as the NO moiety of the nitro group by Tennant et al. (1990) and Ashby (1985), and assigned a + qualification by Benigni (1991).

The results of the NTP bioassay of *o*-nitroanisole in mice and rats have been discussed in greater detail in section 4.0. The positive 2-year bioassay results confirmed the predictions of the QSAR model developed by Benigni (1991) and that of Bakale and McCreary (1992). For a summary of predictive bioassays and NTP results for *o*-nitroanisole, see Omenn et al. (1995).

6.5 Cell Proliferation

Summary: In male and female mice administered 666, 2000, or 6000 ppm *o*-nitroanisole orally for 2 years, focal proliferation of the bronchiolar epithelium was observed, with males exhibiting a higher incidence than females. Hyperplasia of the septal and Bowman's glands and respiratory metaplasia of the olfactory epithelium were significantly increased in females that received the mid- or high-dose and in males that received the high dose. In addition, in all exposed male dose groups, the incidences of eosinophilic foci of the liver were significantly increased. In females dosed with the mid- or high-dose, eosinophilic foci occurred more frequently, but only the incidence in the mid-dose group was significantly increased. In male rats administered 222 or 2000 ppm *o*-nitroanisole orally for 2 years, an increased incidence of focal hyperplasia of the renal tubule epithelium was observed. One female rat fed 2000 ppm *o*-nitroanisole developed focal hyperplasia of the transitional epithelium in the urinary bladder. The incidence of focal hyperplasia in the forestomach increased with exposure level in males and females. In male and female rats fed 6,000 or 18,000 ppm *o*-nitroanisole for up to 27 weeks and then killed 77 weeks after cessation of treatment, there was an increased incidence of urinary bladder squamous metaplasia and connective tissue proliferation. The incidence of hyperplasia of the transitional epithelium of the kidney was also significantly increased in all treated males and females.

In the urinary bladder of mice fed 2500 or 5000 ppm *o*-anisidine hydrochloride in the diet for 103 weeks, hyperplasia was detected in some mice. In the urinary tract of male and female rats fed 5,000 or 10,000 ppm *o*-anisidine hydrochloride in the diet for 103 (low-dose) or 83-88 (high-dose) weeks, epithelial hyperplasia was detected in some rats.

6.5.1 *o*-Nitroanisole

6.5.1.1 Mice

Focal proliferation of the bronchiolar epithelium was observed in male and female mice fed 666, 2000, or 6000 ppm *o*-nitroanisole for 2 years (NTP, 1993; Irwin et al., 1996). However, males showed a greater increased incidence of proliferation (males: 0 ppm, 0/50; 666 ppm, 2/50; 2000 ppm, 13/50; and 6000 ppm, 14/50; females: 0/50; 3/50; 5/50; and 4/50, respectively). Hyperplasia of the septal and Bowman's glands and respiratory metaplasia of the olfactory epithelium were significantly increased in females that received 2000 and 6000 ppm and in males that received 6000 ppm. The respiratory metaplasia of the olfactory epithelium was multifocal in

distribution and often located on the dorsal wall of the dorsal meatus and posterior medial aspects of the nasoturbinates (NTP, 1993; Irwin et al., 1996). In all exposed male dose groups, the incidences of eosinophilic foci of the liver were significantly increased ($p \leq 0.01$; logistic regression test). In females dosed with 2000 and 6000 ppm, eosinophilic foci occurred more frequently, but only the incidence in the 2,000 ppm group was significantly increased ($p \leq 0.05$; logistic regression test).

6.5.1.2 Rats

In male rats fed 222 ppm and 2000 ppm *o*-nitroanisole in feed for 103 weeks, an increased incidence of focal hyperplasia of the renal tubule epithelium was observed (NTP, 1993; Irwin et al., 1996). In the same study, 1 female rat fed 2000 ppm *o*-nitroanisole for 103 weeks developed focal hyperplasia of the transitional epithelium in the urinary bladder, and the incidence of focal hyperplasia in the forestomach increased with exposure level (222, 666, or 2000 ppm) in males and females.

Male and female rats were fed 6,000 or 18,000 ppm *o*-nitroanisole in feed *ad libitum* for up to 27 weeks in a stop-exposure study conducted during the NTP carcinogenesis bioassays (NTP, 1993; Irwin et al., 1996). Groups of 1 to 10 rats from each dose group were killed at 13, 28, 45, or 60 mo for interim evaluation and the remaining rats were killed 77 weeks after cessation of the treatment. An overall increased incidence of urinary bladder neoplasms was found in males and females at 104 weeks: squamous metaplasias (males: low dose, 3/27; high dose, 30/34 vs. control, 0/21; females: low dose, 6/20; high dose, 25/34 vs. control, 0/20) and connective tissue proliferation (males: low dose, 1/27; high dose, 24/34 vs. control, 0/21; females: low dose, 11/20; high dose, 20/34 vs. control, 0/20). The incidence of hyperplasia of the transitional epithelium of the kidney was significantly increased in all treated males (controls, 5/60; low dose, 34/60; and high dose, 27/60) and females (0/60; 6/60; and 19/60, respectively).

6.5.2 *o*-Anisidine Hydrochloride

6.5.2.1 Mice

In the urinary bladder of $B6C3F_1$ mice fed 2500 or 5000 ppm *o*-anisidine hydrochloride in the diet for 103 weeks, hyperplasia was detected in 2/55 low-dose and 21/53 high-dose males (vs. 1/48 controls) and in 1/51 low-dose and 12/50 high-dose females (vs. 0/50 controls). Statistical significance was not specified (NCI, 1978; cited by IARC, 1982).

6.5.2.2 Rats

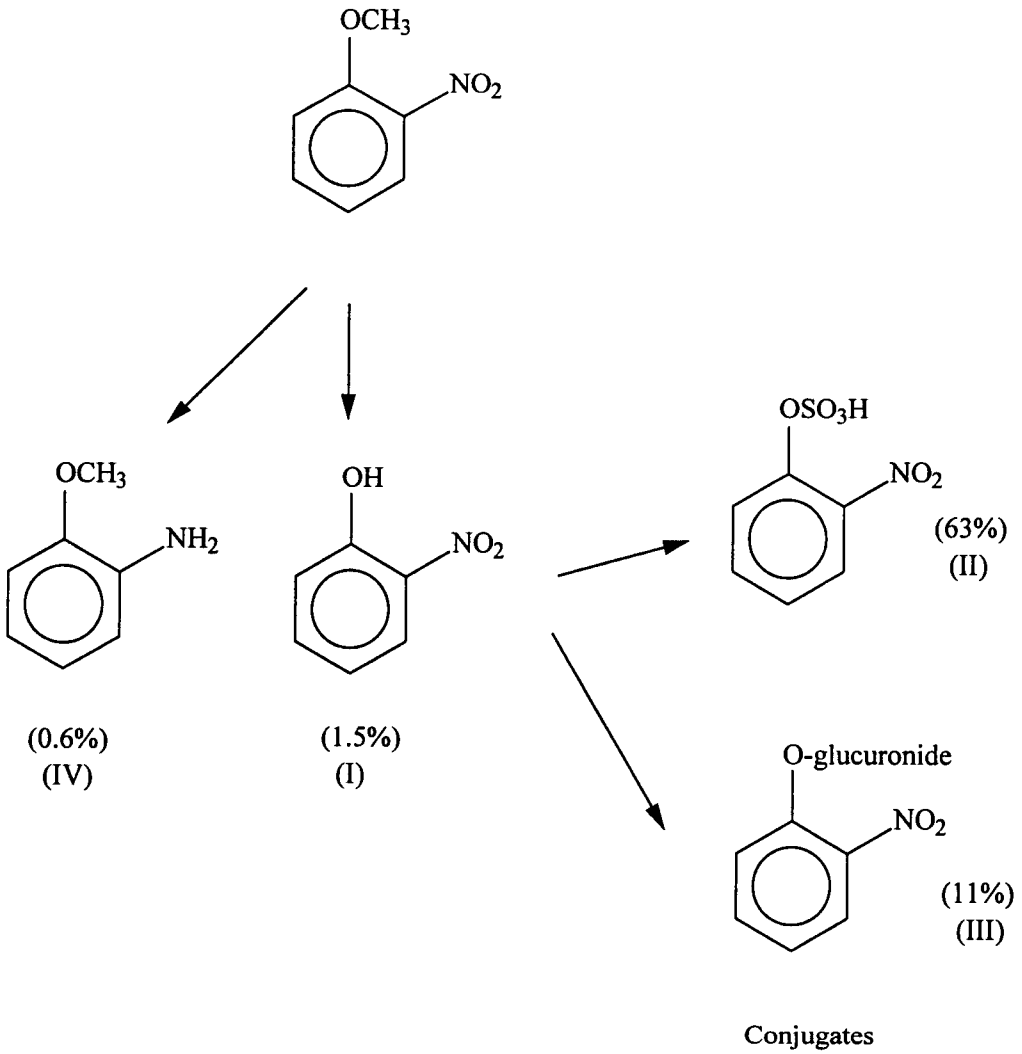
In the urinary tract of male and female F344 rats fed 5,000 or 10,000 ppm *o*-anisidine hydrochloride in the diet for 103 (low-dose) or 83-88 (high-dose) weeks, epithelial hyperplasia was detected in some rats (incidences not given) (NCI, 1978; cited by IARC, 1982).

Table 6-1. *o*-Nitroanisole Metabolite Identification

Metabolite/ *Roman Numeral	Species/Dose	Enzymes/Reaction/Comments	Reference
o-Nitrophenol (I)	F-344 rats orally administered 50 or 500 mg/kg bw (326 or 3260 µmol/kg bw) [^{14}C]*o*-nitroanisole. F-344 rats i.p. administered 25 mg/kg bw (163 µmol/kg bw) [^{14}C]*o*-nitroanisole.	*O*-Demethylation of *o*-nitroanisole forming I (oral administration, 5 to 8% of urinary radioactivity; see also section 6.1). A urinary metabolite profile similar that observed for oral administration was found after i.p. administration of *o*-nitroanisole: II (63%), III (11%), I (1.5%), IV (0.6%).	Miller et al. (1985)
o-Nitrophenyl sulfate (II)		Sulfation of I forming II (oral administration, 64 to 68% of urinary radioactivity; see also section 6.1).	
Nitrophenyl glucuronide (III)		Glucuronidation of II forming III (oral administration, 13 to 15% of urinary radioactivity; see also section 6.1).	
o-Anisidine (IV)		Nitroreduced (oral administration, 0.6% urinary radioactivity; see also section 6.1).	

Abbreviations: bw = body weight; i.p. = intraperitoneal

Figure 6-1. Urinary Metabolites of *o*-Nitroanisole



Source: Miller et al. (1985)

Table 6-2. Cell Proliferation Induced by *o*-Nitroanisole

Age, Strain, and Species	No./Sex Exposed	Controls	Chemical Form, Purity	Dose	Duration of Exposure	Results/Comments	Reference(s)
Mice							
6- to 7-wk-old $B6C3F_1$	10M, 10F per dose	10M, 10F (basal diet alone)	*o*-nitroanisole, > 99% pure	60, 200, 600, 2000, or 6000 ppm in diet	94 days (males) 95 days (females)	Mice were killed at the end of the treatment period. Complete histopathologic examinations were performed on all controls, on all HD mice, and on all mice that died early. **Liver:** Positive (for proliferative activity, as indicated by the presence of hepatocyte hypertrophy; males only, at 200-ppm dose and above) There was a dose-dependent increase in the incidence of hepatocyte hypertrophy in males (0/10 [60 ppm], 3/9 [200 ppm], 9/10 [600 ppm], 10/10 [2000 ppm], 10/10 [6000 ppm] vs. 0/10 controls). Statistical analysis was not performed.	NTP (1993)
40-day-old $B6C3F_1$	60M, 60F per dose	60M, 60F (basal diet alone)	*o*-nitroanisole, > 99% pure	666, 2000, or 6000 ppm in diet	103 wk	Mice were killed either at the end of the treatment period or during a 15-month interim evaluation. Complete histopathologic examinations were performed on all mice. **Nasal Mucosa:** Positive (for proliferative activity, as indicated by presence of glandular hyperplasia; MD and HD only) The incidence of glandular hyperplasia was significantly increased in HD males and in MD and HD females at the 15-month interim evaluation and in MD and HD males and females at the end of the 103-week study (15 months: 10/10 HD males vs. 0/10 male controls, 6/10 MD females [$p \leq 0.05$], 10/10 HD females [$p \leq 0.01$] vs. 0/10 female controls; 103 weeks: 12/50 MD and 49/50 HD males vs. 1/50 male controls [$p \leq 0.01$], 34/50 MD and 50/50 HD females vs. 2/50 female controls [$p \leq 0.01$]). The Fisher exact test and the logistic regression test were used for statistical analysis at the 15-month interim evaluation and at the end of the 103-week study, respectively. **Lungs:** Positive (for proliferative activity, as indicated by presence of focal proliferation of the bronchiolar epithelium; all doses) At the end of the 103-week study, focal proliferation of the bronchiolar epithelium was detected in *o*-nitroanisole-treated mice, but not in controls (2/50 LD, 13/50 MD, and 14/50 HD males vs. 0/50 controls; 3/50 LD, 5/50 MD, and 4/50 HD females vs. 0/50 controls). Statistical analysis was not performed.	NTP (1993); Irwin et al. (1996)

Table 6-2. Cell Proliferation Induced by *o*-Nitroanisole (Continued)

Age, Strain, and Species	No./Sex Exposed	Controls	Chemical Form, Purity	Dose	Duration of Exposure	Results/Comments	Reference(s)
Rats							
5- to 6-wk-old F344	10M, 10F per dose	10M, 10F (basal diet alone)	*o*-nitroanisole, > 99% pure	200, 600, 2000, 6000, or 18,000 ppm in diet	90 days (males) 91 days (females)	Rats were killed at the end of the treatment period. Complete histopathologic examinations were performed on all controls, on all 6000-ppm rats, and on all rats that died early. **Urinary Bladder:** Positive (for proliferative activity, as indicated by presence of hyperplasia; 6000- and 18,000-ppm doses only) All rats that received 6000 or 18,000 ppm *o*-nitroanisole developed urinary bladder hyperplasia (10/10 6000-ppm males, 10/10 18,000-ppm males, 10/10 6000-ppm females, and 10/10 18,000-ppm females, vs. 0/10 male controls and 0/10 female controls [$p \leq 0.01$, Fisher exact test]). The hyperplasia was generally more severe in females than in males and more severe in the 18,000-ppm group than in the 6000-ppm group. None of the rats that received lower doses of *o*-nitroanisole developed urinary bladder hyperplasia. **Spleen:** Positive (for proliferative activity, as indicated by presence of capsular hyperplasia; 18,000-ppm dose only) The incidence of capsular hyperplasia was significantly increased in rats that received 18,000 ppm *o*-nitroanisole (10/10 males, 10/10 females vs. 0/10 male controls and 0/10 female controls; $p \leq 0.01$, Fisher exact test). **Liver:** Positive (for proliferative activity, as indicated by presence of hepatocyte hypertrophy; 18,000-ppm dose only) All rats that received 18,000 ppm *o*-nitroanisole developed hepatocyte hypertrophy (10/10 18,000-ppm males and 10/10 18,000-ppm females, vs. 0/10 male controls and 0/10 female controls [$p \leq 0.01$, Fisher exact test]). None of the rats that received lower doses of *o*-nitroanisole developed hepatocyte hypertrophy.	NTP (1993)

Table 6-2. Cell Proliferation Induced by *o*-Nitroanisole (Continued)

Age, Strain, and Species	No./Sex Exposed	Controls	Chemical Form, Purity	Dose	Duration of Exposure	Results/Comments	Reference(s)
41-day-old F344	60M, 60F per dose 10M and 10F were examined at the 3, 6, 9, and 15-mo interim evaluations	60M, 60F (basal diet alone)	*o*-nitroanisole, > 99% pure	6000 or 18,000 ppm in diet	27 wk	After the 27-week treatment period, rats were held until the termination of the study, 77 weeks after the end of the 27-week treatment period (104 wk total). Ten rats/group were scheduled for interim evaluations after 3, 6, 9, and 15 months. At the interim evaluations and at the terminal sacrifice, kidneys, liver, spleen, testes (including epididymis), urinary bladder, ureter, uterus, and gross lesions were examined microscopically in all treatment groups. Statistical analysis was performed using the Fisher exact test. All HD males and females died before the 15-month interim evaluation. **Urinary Bladder:** Positive (for proliferative activity, as indicated by presence of proliferation of connective tissue of lamina propria and hyperplasia of transitional epithelium) The incidence of proliferation of connective tissue in the lamina propria (characterized by scattered inflammatory cells, principally neutrophils and macrophages, and increased numbers of fibroblasts with immature collagen) was significantly increased in HD males and females at the 3-, 6-, and 9-month evaluations and in HD males and MD and HD females at the study termination (3 months: 10/10 HD males vs. 0/9 controls [$p \leq 0.01$], 6/10 HD females vs. 0/10 controls [$p \leq 0.01$]; 6 months: 9/10 HD males vs. 0/10 controls [$p \leq 0.01$], 10/10 HD females vs. 0/10 controls [$p \leq 0.01$]; 9 months: 4/6 HD males vs. 0/10 controls [$p \leq 0.01$], 4/6 HD females vs. 1/9 controls [$p \leq 0.01$]; 2 years: 24/34 HD males vs. 0/21 controls [$p \leq 0.01$], 11/20 MD and 20/34 HD females vs. 0/20 controls [$p \leq 0.01$]). The incidence of hyperplasia of the transitional epithelium was significantly increased in HD males and in MD and HD females at the 3-month interim evaluation, in MD males and females at the 6- and 9-month interim evaluations, and in MD males at the study termination (3 months: 9/10 HD males vs. 0/9 controls [$p \leq 0.01$], 8/10 MD and 10/10 HD females vs. 0/10 controls [$p \leq 0.01$]; 6 months: 10/10 MD males vs. 0/10 controls [$p \leq 0.01$], 10/10 MD females vs. 0/10 controls [$p \leq 0.01$]; 9 months: 9/10 MD males vs. 0/10 controls [$p \leq 0.01$], 9/9 MD females vs. 0/10 controls [$p \leq 0.01$]; 2 years: 9/27 MD males vs. 0/21 controls [$p \leq 0.01$]). **Kidneys:** Positive (for proliferative activity, as indicated by presence of hyperplasia of transitional cell epithelium) The incidence hyperplasia of the transitional epithelium was significantly increased in HD males at the 6-month interim evaluation, in MD and HD males at the 9-month interim evaluation, and in MD and HD males and females at the study termination (6 months: 5/10 HD males vs. 0/10 controls [$p \leq 0.05$]; 9 months: 7/10 MD [$p \leq 0.01$] and 3/6 HD males [$p \leq 0.05$] vs. 0/10 controls; 2 years: 24/27 MD [$p \leq 0.01$] and 19/34 HD [$p \leq 0.05$] males vs. 5/21 controls; 5/20 MD [$p \leq 0.05$] and 16/34 HD [$p \leq 0.01$] females vs. 0/22 controls).	NTP (1993); Irwin et al. (1996)

Table 6-2. Cell Proliferation Induced by *o*-Nitroanisole (Continued)

Age, Strain, and Species	No./Sex Exposed	Controls	Chemical Form, Purity	Dose	Duration of Exposure	Results/Comments	Reference(s)
41-day-old F344 (cont'd.)	60M, 60F per dose 10M and 10F were examined at the 3, 6, 9, and 15-mo interim evaluations	60M, 60F (basal diet alone)	*o*-nitroanisole, > 99% pure	6000 or 18,000 ppm in diet	27 wk	**Liver:** Positive (for proliferative activity, as indicated by presence of centrilobular hypertrophy; at 3- and 6-month interim evaluations only) The incidence of centrilobular hypertrophy was significantly increased in HD males and females at the 3- and 6-month interim evaluations, but not at the 9-month interim evaluation (no HD rats survived until the 15-month interim evaluation) or at the study termination (3 months: 10/10 HD males vs. 0/10 controls [$p \leq 0.01$], 10/10 HD females vs. 0/10 controls [$p \leq 0.01$]; 6 months: 10/10 HD males vs. 0/10 controls [$p \leq 0.01$], 10/10 HD females vs. 0/10 controls [$p \leq 0.01$]). **Spleen:** Positive (for proliferative activity, as indicated by presence of capsular hypertrophy and inflammation) The incidence of capsular hypertrophy was significantly increased in MD and HD males and females at the 3-, 6-, and 9-month interim evaluations and at the study termination and in MD males and females at the 15-month interim evaluation (no HD rats survived until the 15-month interim evaluation) (3 months: 10/10 MD and 10/10 HD males vs. 0/10 controls [$p \leq 0.01$], 10/10 MD and 10/10 HD females vs. 0/10 controls [$p \leq 0.01$]; 6 months: 7/10 MD and 10/10 HD males vs. 0/10 controls [$p \leq 0.01$], 10/10 MD and 10/10 HD females vs. 0/10 controls [$p \leq 0.01$]; 9 months: 8/10 MD and 6/6 HD males vs. 0/10 controls [$p \leq 0.01$], 8/10 MD and 6/6 HD females vs. 0/10 controls [$p \leq 0.01$]; 15 months: 2/3 MD males vs. 0/9 controls [$p \leq 0.05$], 9/10 MD females vs. 0/8 controls [$p \leq 0.01$]; 2 years: 15/27 MD and 34/34 HD males vs. 0/21 controls [$p \leq 0.01$], 9/20 MD and 33/34 HD females vs. 0/22 controls [$p \leq 0.01$]). The incidence of capsular inflammation was significantly increased in HD males and in MD and HD females at the 3-month interim evaluation, in HD males and females at the 6- and 9-month interim evaluations, and in MD and HD males and HD females at the study termination (3 months: 10/10 HD males vs. 0/10 controls [$p \leq 0.01$], 6/10 MD and 9/10 HD females vs. 0/10 controls [$p \leq 0.01$]; 6 months: 10/10 HD males vs. 0/10 controls [$p \leq 0.01$], 10/10 HD females vs. 0/10 controls [$p \leq 0.01$]; 9 months: 6/6 HD males vs. 0/10 controls [$p \leq 0.01$], 6/6 HD females vs. 0/10 controls [$p \leq 0.01$]; 2 years: 5/27 MD [$p \leq 0.05$] and 33/34 HD [$p \leq 0.01$] males vs. 0/21 controls, 30/34 HD females vs. 0/22 controls [$p \leq 0.01$]).	NTP (1993); Irwin et al. (1996)

Table 6-2. Cell Proliferation Induced by *o*-Nitroanisole (Continued)

Age, Strain, and Species	No./Sex Exposed	Controls	Chemical Form, Purity	Dose	Duration of Exposure	Results/Comments	Reference(s)
40-day-old F344	60M, 60F per dose	60M, 60F (basal diet alone)	*o*-nitroanisole, > 99% pure	222, 666, or 2000 ppm in diet	103 wk	Rats were killed either at the end of the treatment period or during a 15-month interim evaluation. Complete histopathologic examinations were performed on all controls, on all HD rats, on all rats that died early, and on rats killed at the 15-month interim evaluation. In LD and MD rats, the clitoral gland, epididymis, kidneys, liver, preputial gland, spleen, testes, urinary bladder, and uterus were examined. **Liver:** Positive (for proliferative activity, as indicated by presence of bile duct hyperplasia [HD females only] and nodular hyperplasia [MD males and HD females only]) The incidence of focal hyperplasia of the bile ducts in the portal areas of the liver lobules was significantly increased at the end of the 103-week study in HD females only (43/50 vs. 29/50 controls; $p \leq 0.01$, logistic regression test). The incidence of nodular hyperplasia was significantly increased at the end of the 103-week study in MD males and in HD females (18/50 MD [$p \leq 0.01$, logistic regression test] and 14/50 HD [not significant] males vs. 7/50 controls; 14/50 HD females vs. 4/50 controls [$p \leq 0.01$, logistic regression test]). The incidence of hyperplasia was not significantly increased in animals examined at the 15-month interim evaluation. **Forestomach:** Positive (for proliferative activity, as indicated by presence of focal hyperplasia; LD, MD, and HD males, HD females) The incidence of focal hyperplasia was significantly increased in all groups of *o*-nitroanisole-treated males and in HD females at the end of the 103-week study (16/50 LD, 25/50 MD, and 32/50 HD males vs. 3/50 controls [$p \leq 0.01$, logistic regression test]; 28/50 HD females vs. 8/50 controls [$p \leq 0.01$, logistic regression test]). The incidence of focal hyperplasia was not significantly increased at the 15-month interim evaluation.	NTP (1993); Irwin et al. (1996)

Abbreviations: F = females; HD = high dose; LD = low dose; M = males; MD = mid dose

Table 6-3. Cell Proliferation Induced by *o*-Nitroanisole Metabolite *o*-Anisidine Hydrochloride

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form, Purity	Dose	Duration of Exposure	Results/Comments	Reference
6-wk-old B6C3F$_1$ mice	55M, 55F per dose	55M, 55F (basal diet alone)	*o*-anisidine hydrochloride, > 99%	2500 or 5000 ppm in diet	103 wk	Surviving mice were killed at 105 weeks. **Urinary Bladder:** Positive (for proliferative activity, as indicated by presence of hyperplasia; HD only) Urinary bladder hyperplasia was detected in 2/55 LD and 21/53 HD males (vs. 1/48 controls) and in 1/51 LD and 12/50 HD females (vs. 0/50 controls). Statistical significance was not specified in the IARC review.	NCI (1978; cited by IARC, 1982)
6-wk-old F344 rats	55M, 55F per dose	55M, 55F (basal diet alone)	*o*-anisidine hydrochloride, > 99%	5000 or 10,000 ppm in diet	103 wk (LD) 83-88 wk (HD)	Surviving rats were killed at 106-107 weeks. **Urinary Tract:** Epithelial hyperplasia of the urinary tract was detected in some *o*-anisidine-treated rats (incidences not given and statistical significance not specified).	NCI (1978; cited by IARC, 1982)

Abbreviations: F = females; HD = high dose; LD = low dose; M = males

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APPENDIX A

DESCRIPTION OF ONLINE SEARCHES FOR *o*-NITROANISOLE

DESCRIPTION OF ONLINE SEARCHES FOR *o*-NITROANISOLE (IARC Monograph in Vol. 65, 1996)

Online searching was done by the technical support contractor in TOXLINE in March 1996, using the CASRN (retrieved 58 records) and the name nitroanisole. IARC (1996) and NTP (1993) were to be relied on for identification of pertinent earlier references; however, at the time of the search, only a rough draft of the IARC monograph was available. The emphasis of the search was to supplement the few studies cited that were published in the 1990s. From the resulting records, after exclusion of *p*-nitroanisole records, the contractor selected approximately 30 for acquisition.

An exhaustive search of other pertinent toxicology databases was not attempted for the nitroarenes. A high degree of redundancy had been noted between TOXLINE and the databases CANCERLIT, EMBASE (Excerpta Medica), MEDLINE, and NIOSHTIC (Occupational Safety and Health). No special attempt was made to find toxicity information about metabolites and other structural analogues in the search strategy.

The contractor also searched CSCHEM and CSCORP for U.S. suppliers (Chem Sources databases); EMIC; EMICBACK; HSDB; IRIS; TSCATS (Toxic Substances Control Act Test Submissions); the Chemical Information System's databases SANSS (the Structure and Nomenclature Search System), ISHOW (for physical-chemical properties), and REGMAT (May 1993 version; this regulatory information database with broad coverage of EPA regulations is no longer available); Chemical Abstracts Service's (CAS) CA and Registry Files for metabolism studies (152 records) and metabolite identification; CAS File CHEMLIST for TSCA and SARA updates in 1996; and CA File sections 59 (Air Pollution and Industrial Hygiene), 60 (Waste Disposal and Treatment), and 61 (Water) for environmental exposure information. For current awareness, the contractor monitored Current Contents on Diskette® Life Sciences 1200 [journals] edition. Older literature that needed to be examined was identified from the reviews and original articles as they were acquired.

APPENDIX B

LISTING OF GAP TEST CODES IN ALPHABETICAL ORDER

LISTING OF GAP TEST CODES IN ALPHABETICAL ORDER

Test Code	Definition
ACC	Allium cepa, chromosomal aberrations
AIA	Aneuploidy, animal cells in vitro
AIH	Aneuploidy, human cells in vitro
ANF	Aspergillus nidulans, forward mutation
ANG	Aspergillus nidulans, genetic crossing-over
ANN	Aspergillus nidulans, aneuploidy
ANR	Aspergillus nidulans, reverse mutation
ASM	Arabidopsis species, mutation
AVA	Aneuploidy, animal cells in vivo
AVH	Aneuploidy, human cells in vivo
BFA	Body fluids from animals, microbial mutagenicity
BFH	Body fluids from humans, microbial mutagenicity
BHD	Binding (covalent) to DNA, human cells in vivo
BHP	Binding (covalent) to RNA or protein, human cells in vivo
BID	Binding (covalent) to DNA in vitro
BIP	Binding (covalent) to RNA or protein in vitro
BPF	Bacteriophage, forward mutation
BPR	Bacteriophage, reverse mutation
BRD	Other DNA repair-deficient bacteria, differential toxicity
BSD	Bacillus subtilis rec strains, differential toxicity
BSM	Bacillus subtilis multi-gene test
BVD	Binding (covalent) to DNA, animal cells in vivo
BVP	Binding (covalent) to RNA or protein, animal cells in vivo
CBA	Chromosomal aberrations, animal bone-marrow cells in vivo
CBH	Chromosomal aberrations, human bone-marrow cells in vivo
CCC	Chromosomal aberrations, spermatocytes treated in vivo and cytes obs.
CGC	Chromosomal aberrations, spermatogonia treated in vivo and cytes obs.
CGG	Chromosomal aberrations, spermatogonia treated in vivo and gonia obs.
CHF	Chromosomal aberrations, human fibroblasts in vitro
CHL	Chromosomal aberrations, human lymphocyte in vitro
CHT	Chromosomal aberrations, transformed human cells in vitro
CIA	Chromosomal aberrations, other animal cells in vitro
CIC	Chromosomal aberrations, Chinese hamster cells in vitro
CIH	Chromosomal aberrations, other human cells in vitro
CIM	Chromosomal aberrations, mouse cells in vitro
CIR	Chromosomal aberrations, rat cells in vitro
CIS	Chromosomal aberrations, Syrian hamster cells in vitro
CIT	Chromosomal aberrations, transformed animal cells in vitro
CLA	Chromosomal aberrations, animal leukocytes in vivo
CLH	Chromosomal aberrations, human lymphocytes in vivo

Test Code	Definition
COE	Chromosomal aberrations, oocytes or embryos treated in vivo
CVA	Chromosomal aberrations, other animal cells in vivo
CVH	Chromosomal aberrations, other human cells in vivo
DIA	DNA strand breaks, cross-links or rel. damage, animal cells in vitro
DIH	DNA strand breaks, cross-links or rel. damage, human cells in vitro
DLM	Dominant lethal test, mice
DLR	Dominant lethal test, rats
DMC	Drosophila melanogaster, chromosomal aberrations
DMG	Drosophila melanogaster, genetic crossing-over or recombination
DMH	Drosophila melanogaster, heritable translocation test
DML	Drosophila melanogaster, dominant lethal test
DMM	Drosophila melanogaster, somatic mutation (and recombination)
DMN	Drosophila melanogaster, aneuploidy
DMX	Drosophila melanogaster, sex-linked recessive lethal mutation
DVA	DNA strand breaks, cross-links or rel. damage, animal cells in vivo
DVH	DNA strand breaks, cross-links or rel. damage, human cells in vivo
ECB	Escherichia coli (or E. coli DNA), strand breaks, cross-links or repair
ECD	Escherichia coli pol A/W3110-P3478, diff. toxicity (spot test)
ECF	Escherichia coli (excluding strain K12), forward mutation
ECK	Escherichia coli K12, forward or reverse mutation
ECL	Escherichia coli pol A/W3110-P3478, diff. toxicity (liquid susp. test)
ECR	Escherichia coli, miscellaneous strains, reverse mutation
ECW	Escherichia coli WP2 uvrA, reverse mutation
EC2	Escherichia coli WP2, reverse mutation
ERD	Escherichia coli rec strains, differential toxicity
FSC	Fish, chromosomal aberrations
FSI	Fish, micronuclei
FSM	Fish, mutation
FSS	Fish, sister chromatid exchange
FSU	Fish, unscheduled DNA synthesis
GCL	Gene mutation, Chinese hamster lung cells exclusive of V79 in vitro
GCO	Gene mutation, Chinese hamster ovary cells in vitro
GHT	Gene mutation, transformed human cells in vivo
GIA	Gene mutation, other animal cells in vitro
GIH	Gene mutation, human cells in vitro
GML	Gene mutation, mouse lymphoma cells exclusive of L5178Y in vitro
GVA	Gene mutation, animal cells in vivo
G5T	Gene mutation, mouse lymphoma L5178Y cells in vitro, TK locus
G51	Gene mutation, mouse lymphoma L5178Y cells in vitro, all other loci
G9H	Gene mutation, Chinese hamster lung V-79 cells in vitro, HPRT locus
G9O	Gene mutation, Chinese hamster lung V-79 cells in vitro, ouabain resistance
HIM	Haemophilus influenzae, mutation
HMA	Host mediated assay, animal cells in animal hosts

Test Code	Definition
HMH	Host mediated assay, human cells in animal hosts
HMM	Host mediated assay, microbial cells in animal hosts
HSC	Hordeum species, chromosomal aberrations
HSM	Hordeum species, mutation
ICH	Inhibition of intercellular communication, human cells in vitro
ICR	Inhibition of intercellular communication, rodent cells in vitro
KPF	Klebsiella pneumonia, forward mutation
MAF	Micrococcus aureus, forward mutation
MHT	Mouse heritable translocation test
MIA	Micronucleus test, animal cells in vitro
MIH	Micronucleus test, human cells in vitro
MST	Mouse spot test
MVA	Micronucleus test, other animals in vivo
MVC	Micronucleus test, hamsters in vivo
MVH	Micronucleus test, human cells in vivo
MVM	Micronucleus test, mice in vivo
MVR	Micronucleus test, rats in vivo
NCF	Neurospora crassa, forward mutation
NCN	Neurospora crassa, aneuploidy
NCR	Neurospora crassa, reverse mutation
PLC	Plants (other), chromosomal aberrations
PLI	Plants (other), micronuclei
PLM	Plants (other), mutation
PLS	Plants (other), sister chromatid exchanges
PLU	Plants, unscheduled DNA synthesis
PRB	Prophage, induction, SOS repair, DNA strand breaks, or cross-links
PSC	Paramecium species, chromosomal aberrations
PSM	Paramecium species, mutation
RIA	DNA repair exclusive of UDS, animal cells in vitro
RIH	DNA repair exclusive of UDS, human cells in vitro
RVA	DNA repair exclusive of UDS, animal cells in vivo
SAD	Salmonella typhimurium, DNA repair-deficient strains, differential toxicity
SAF	Salmonella typhimurium, forward mutation
SAL	Salmonella typhimurium, all strains, reverse mutation
SAS	Salmonella typhimurium (other misc. strains), reverse mutation
SA0	Salmonella typhimurium TA100, reverse mutation
SA1	Salmonella typhimurium TA97, reverse mutation
SA2	Salmonella typhimurium TA102, reverse mutation
SA3	Salmonella typhimurium TA1530, reverse mutation
SA4	Salmonella typhimurium TA104, reverse mutation
SA5	Salmonella typhimurium TA1535, reverse mutation
SA7	Salmonella typhimurium TA1537, reverse mutation
SA8	Salmonella typhimurium TA1538, reverse mutation

Test Code	Definition
SA9	Salmonella typhimurium TA98, reverse mutation
SCF	Saccharomyces cerevisiae, forward mutation
SCG	Saccharomyces cerevisiae, gene conversion
SCH	Saccharomyces cerevisiae, homozygosis by recombination or gene conversion
SCN	Saccharomyces cerevisiae, aneuploidy
SCR	Saccharomyces cerevisiae, reverse mutation
SGR	Streptomyces griseoflavus, reverse mutation
SHF	Sister chromatid exchange, human fibroblasts in vitro
SHL	Sister chromatid exchange, human lymphocytes in vitro
SHT	Sister chromatid exchange, transformed human cells in vitro
SIA	Sister chromatid exchange, other animal cells in vitro
SIC	Sister chromatid exchange, Chinese hamster cells in vitro
SIH	Sister chromatid exchange, other human cells in vitro
SIM	Sister chromatid exchange, mouse cells in vitro
SIR	Sister chromatid exchange, rat cells in vitro
SIS	Sister chromatid exchange, Syrian hamster cells in vitro
SIT	Sister chromatid exchange, transformed cells in vitro
SLH	Sister chromatid exchange, human lymphocytes in vivo
SLO	Mouse specific locus test, other stages
SLP	Mouse specific locus test, postspermatogonia
SPF	Sperm morphology, F1 mouse
SPH	Sperm morphology, human
SPM	Sperm morphology, mouse
SPR	Sperm morphology, rat
SPS	Sperm morphology, sheep
SSB	Saccharomyces species, DNA breaks, cross-links or related damage
SSD	Saccharomyces cerevisiae, DNA repair-deficient strains, diff. toxicity
STF	Streptomyces coelicolor, forward mutation
STR	Streptomyces coelicolor, reverse mutation
SVA	Sister chromatid exchange, animal cells in vivo
SVH	Sister chromatid exchange, other human cells in vivo
SZD	Schizosaccharomyces pombe, DNA repair-deficient strains, diff. toxicity
SZF	Schizosaccharomyces pombe, forward mutation
SZG	Schizosaccharomyces pombe, gene conversion
SZR	Schizosaccharomyces pombe, reverse mutation
T7R	Cell transformation, SA7/rat cells
T7S	Cell transformation, SA7/Syrian hamster embryo cells
TBM	Cell transformation, BALB/C3T3 mouse cells
TCL	Cell transformation, other established cell lines
TCM	Cell transformation, C3H10T1/2 mouse cells
TCS	Cell transformation, Syrian hamster embryo cells, clonal assay
TEV	Cell transformation, other viral enhancement systems
TFS	Cell transformation, Syrian hamster embryo cells, focus assay

Test Code	Definition
TIH	Cell transformation, human cells in vitro
TPM	Cell transformation, mouse prostate cells
TRR	Cell transformation, RLV/Fischer rat embryo cells
TSC	Tradescantia species, chromosomal aberrations
TSI	Tradescantia species, micronuclei
TSM	Tradescantia species, mutation
TVI	Cell transformation, treated in vivo, scored in vitro
UBH	Unscheduled DNA synthesis, human bone-marrow cells in vivo
UHF	Unscheduled DNA synthesis, human fibroblasts in vitro
UHL	Unscheduled DNA synthesis, human lymphocytes in vitro
UHT	Unscheduled DNA synthesis, transformed human cells in vitro
UIA	Unscheduled DNA synthesis, other animal cells in vitro
UIH	Unscheduled DNA synthesis, other human cells in vitro
UPR	Unscheduled DNA synthesis, rat hepatocytes in vivo
URP	Unscheduled DNA synthesis, rat primary hepatocytes
UVA	Unscheduled DNA synthesis, other animal cells in vivo
UVC	Unscheduled DNA synthesis, hamster cells in vivo
UVH	Unscheduled DNA synthesis, other human cells in vivo
UVM	Unscheduled DNA synthesis, mouse cells in vivo
UVR	Unscheduled DNA synthesis, rat cells (other than hepatocytes) in vivo
VFC	Vicia faba, chromosomal aberrations
VFS	Vicia faba, sister chromatid exchange

APPENDIX C

REGULATIONS TABLE FOR *o*-NITROANISOLE

REGULATIONS TABLE FOR *o*-NITROANISOLE

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 60—PART 60—STANDARDS OF PERFORMANCE FOR NEW STATIONARY SOURCES. Promulgated: 36 FR 24877, 12/31/71.	The provisions of this part apply to the owner/operator of any stationary source which contains an affected facility (a stationary source with an apparatus to which a standard is applicable).
	40 CFR 60.489—Sec. 60.489 List of chemicals produced by affected facilities.	
	40 CFR 63—PART 63—NATIONAL EMISSION STANDARDS FOR HAZARDOUS AIR POLLUTANTS FOR SOURCE CATEGORIES. Promulgated: 57 FR 61992, 12/29/92. U.S. Code: 7401 et seq.	Standards that regulate specific categories of stationary sources that emit (or have the potential to emit) one or more hazardous air pollutants are listed in this part pursuant to section 112(b) of the CAA.
	30 CFR 63.100 ff.—Subpart F—National Emission Standard for Organic Hazardous Air Pollutant From the Syntheitc Organic Chemical Manufacturing Industry. Promulgated: 59 FR 19454, 04/22/94.	Owners and operators of sources subject to this subpart shall comply with the requirements of subparts G and H of this part. This subpart applies to chemical manufacturing process units that manufacture one or more of the chemical listed in Table 1 and Table 2 of this subpart and are located at a plant site that is a major source as defined in section 112(a) of CAA.

[a] The regulations in this table have been updated through the 1996 Code of Federal Regulations: 40 CFR, July 1, 1996; 21 CFR, July 1, 1996; 29 CFR, July 1, 1996.